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Effect of the Combined Action of Selenium and Arsenic on Suspension Culture of Mice Fibroblasts

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The protective influence of arsenic against the toxic action of selenium has been tested on suspension cultures of mice fibroblasts LA 115. The growth of the cells was observed after the isolated and combined action of sodium arsenite $NaAsO_2$ and sodium selenite Na_2SeO_3. The concentration range of both substances in cultivation medium MEm (ÚSOL) was $10^{-5} - 10^{-11} M$. The growth of treated cultures was analyzed daily during 5 days of exposure. From the results obtained, growth curves of the cell cultures were constructed and analyzed. The results of every determination were evaluated in relation to the corresponding control culture.

The results obtained demonstrate that decreasing concentrations of arsenic enhanced its protective effect in the range of the concentrations used. In contrast, a low protective effect of selenium against arsenic was noted in the concentrations employed. The cell cultures have proved to be very suitable for toxicological studies of the combined effects of different substances.

The isolated action of toxic agents in the living as well as working environment of man is a rather rare phenomenon. Therefore, the combined action of these substances is of increasing importance in environmental studies. From the general toxicological aspect there is either a summation, potentiation or, conversely, a depression of the toxic effect of one toxic agent in the presence of another one. The antagonism of arsenic and selenium, with which we are concerned, has been known since 1938 (*1*). It has been used in practice to protect domestic animals from chronic intoxication with selenium occurring in large amounts in fodder in some regions. If the animals are given drinking water containing 5–10 mg arsenic/l., selenium poisoning does not develop (*2*). In our study we tried to ascertain whether the same protective effect applies also to cells cultivated *in vitro* in cell cultures. The design of our study was based on previous experience gained with the isolated action of both toxic elements (*3–5*).

The experiment was performed on suspension culture of La 115 mice fibroblasts. The cells were in their logarithmic growth phase. The Müller cultivation bottles contained 1×10^6 cells in 10 ml medium. A MEM cultivation medium (ÚSOL) was supplemented with 5% calf serum, 0.5% sodium, and 1% ATB (antibiotics, 100 IU PNC, 100μg STM/ml). The growth of the cells was observed after the isolated and combined action of sodium arsenite $NaAsO_2$ and sodium selenite Na_2SeO_3 5 H_2O (Merck). The concentration of these substances in the cultivation medium was chosen on the basis of previous experience in the range 10^{-5} to $10^{-11} M$, i.e. 1.3 (2.63) μg/ml to 1.3 (2.63) $\times 10^{-6}$ μg/ml. In order to ensure realability of the results two parallel cultures were used and the experiments were repeated three times. Changes in the cell count in 1 ml medium were determined in 24-hr intervals by counting in the Bürker chamber. From the results obtained, a growth curve of the cell cultures during the 5 days of exposure to arsenic and selenium was constructed. The results of every determination were evaluated in relation to the corresponding control culture.

Figure 1 shows the growth of cells in relation to growth of the control with time after exposure to Se and/or As. The control represents 100% and is illustrated by a dotted line. Figure 1 indicates that both Se and As alone at concentrations of $10^{-7} M$ were nontoxic and their values were similar to those of

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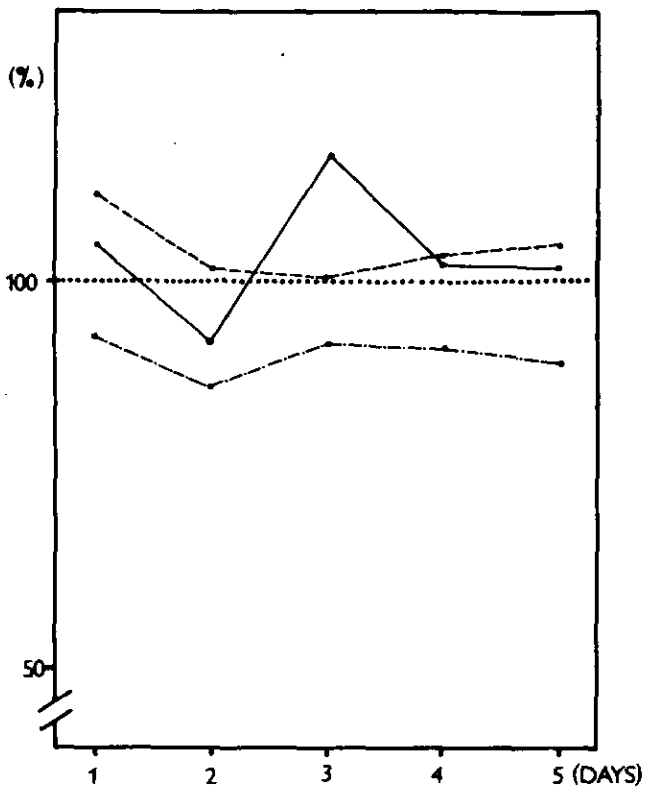

FIGURE 1. *Growth of cells in preparations treated with As and/or Se relative to control preparation: (. . .) control; (——) $10^{-7}M$ Se; (– –) $10^{-7}M$ As; (–·–·) $10^{-7}M$ Se + $10^{-7}M$ As.*

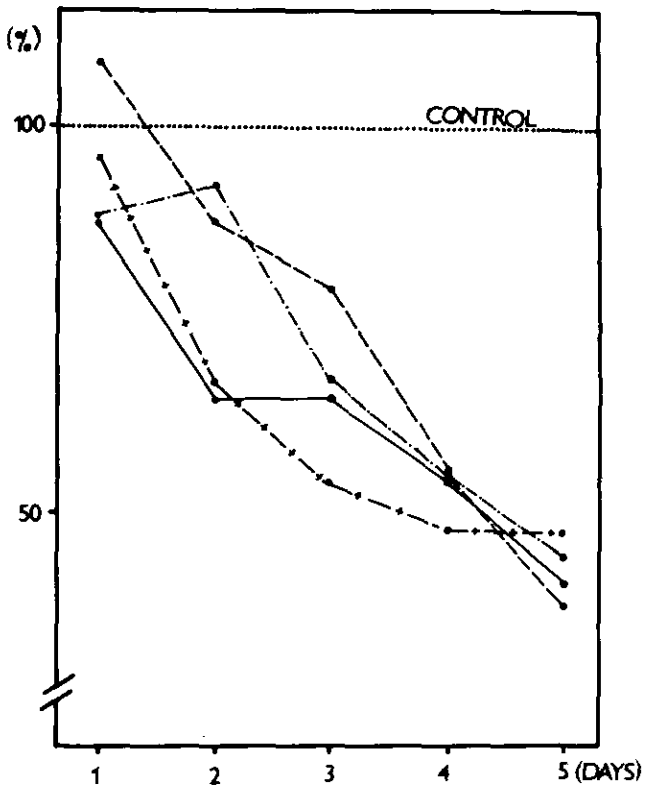

FIGURE 2. *Growth of cells in preparations treated with As and/or Se relative to control preparation: (. . .) control; (——) $10^{-5}M$ As; (– –) $10^{-5}M$ As + $10^{-5}M$ Se; (– · –) $10^{-5}M$ As + $10^{-6}M$ Se; (—+) $10^{-5}M$ As + $10^{-7}M$ Se.*

the control. Simultaneous lower concentrations manifested no effect and higher concentrations were also singly toxic. At a simultaneous action the values did not reach more than 90% of the control. This result is interesting, even though the deviation of the curve from the control was not statistically significant.

Figure 2 shows that the effect of the toxic concentration of arsenic at $10^{-5}M$ is modified by different concentrations of selenium. A diminished toxic effect of arsenic can be achieved by a toxic concentration of Se of $10^{-5}M$ in the first 3 days of growth. The nontoxic concentration of Se $10^{-7}M$ remained without effect.

Figure 3 shows clearly the protective effect of As (III) against the toxic concentration of Se of $10^{-5}M$. The effect was marked in a combination with nontoxic concentrations of As of 10^{-7} or $10^{-9}M$ for a proliferating activity of cells near that of the control in the first 3 days. The toxic concentration of As of $10^{-5}M$ had no protective influence.

In conclusion it can be said that the hypothesis of the protective influence of arsenic against the toxic action of selenium has been confirmed. The results demonstrate that decreasing concentration of arsenic enhance its protective effect in the range of concentrations used. In contrast, a low protective effect of selenium against arsenic was noted in the concentrations employed.

Cell cultures have again proved to be extremely suitable for toxicological model studies of the combined effect of substances.

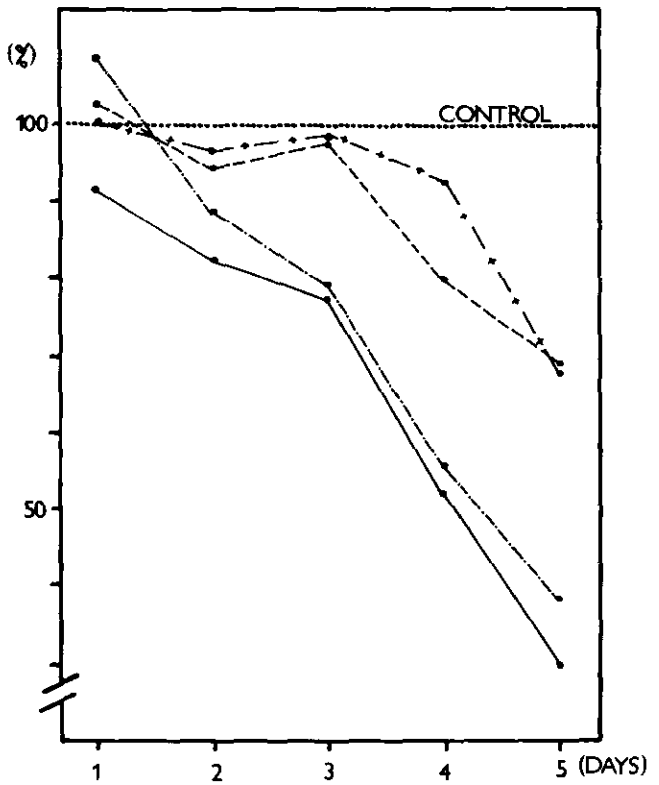

FIGURE 3. Growth of cells in preparations treated with As and/or Se relative to control preparation: (. . .) control; (——) $10^{-5}M$ Se; (– · –) $10^{-5}M$ Se + $10^{-5}M$ As; (– –) $10^{-5}M$ Se + $10^{-7}M$ As; (–+) $10^{-5}M$ Se + $10^{-9}M$ As.

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